RAISED PLASMA KININOGEN LEVELS IN RHEUMATOID PATIENTS - RESPONSE TO THERAPY WITH NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

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In the aetiology of rheumatoid arthritis there is some evidence to implicate the formation of kinins in synovial fluid (Melmon, Webster, Goldfinger & Seegmiller, 1967; Keele & Eisen, 1970). The relationship between synovial kinin levels and clinical symptoms is, however, inconsistent (Keele & Eisen, 1970; Webster & Maling, 1970). In the systemic circulation no abnormalities in the kinin system have previously been described in this disease. We have recently reported some preliminary findings concerning changes in plasma kininogen levels in rheumatoid patients (Brooks, Dick, Sharma & Zeitlin, 1974; Zietlin, Sharma, Brooks & Dick, In Press). When patients with active rheumatoid arthritis received no treatment for 48 hours, their mean venous kininogen levels rose to more than twice the level found in eleven healthy volunteers or in five non-inflamed convalescent fracture patients. When the rheumatoid patients were put back on indomethacin therapy for one week, their mean plasma kininogen level fell to nearly half of the untreated value and lay within the healthy control range.

We have now examined the time course of the kininogen and plasma protein changes produced by indomethacin and report that aspirin produces similar changes. Some of these results were reported at the Symposium in Future Trends in Inflammation (Paris, 1975).

METHODS

Fifteen patients with seropositive rheumatoid arthritis (age range 26 - 74 years) consented to take part in the study. Four male and 8 female patients were involved in the indomethacin study,

6 female patients took part in the aspirin study.

At the start of each test, therapy was stopped for 48 hours and replaced by placebo indistinguishable from the drug to be used in the test. At the end of 48 hours of placebo, blood was sampled from an arm vein for the estimation of plasma kininogen, plasma proteins, PCV and plasma drug levels. Clinical assessment was carried out by a single blind observer. The activity of the disease was monitored by measuring joint diameter, grip strength, an articular index of joint tenderness produced by squeezing or manipulating some 26 joints and a pain index based on the patients own assessment on a 0 - 4 scale (Lee, Webb, Anderson & Buchanan, 1973; Ritchie, Boyle, McInnes, Jasani, Dalakos, Greiveson, Buchanan, 1973). The patients then commenced therapy with the test drug.

Six patients were given oral indomethacin, 50 mg thrice daily for seven days followed by one further day on placebo. In three of these patients and in an additional three the effect was determined of a single 50 mg dose of indomethacin on the biochemical parameters A further six patients received 975 mg of enteric coated aspirin, four times a day for one week.

Plasma kininogen levels were measured using the method of Brocklehurst and Zeitlin (1967) involving ethanolic precipitation of the kininogen protein which was ultimately activated using trypsin. Bradykinin-like activity was assayed using the isolated oestrus rat uterus. Kininogen concentrations are stated as microgrammes bradykinin equivalent per ml. of plasma (µg Bk Eq per ml) giving mean and standard deviation.

Indomethacin was measured fluorimetrically after extraction from plasma (Emori, Champion, Bluestone, Paulus, 1973).

Plasma proteins were measured using cellulose acetate electrophoresis and scanning densitometry.

Statistical significance of differences was tested using the paired t-test and the Mann-Whitney 'U'-test for small numbers.

RESULTS

Plasma Kininogen Levels in Untreated Rheumatoids

In fifteen rheumatoid patients after 48 hours of placebo administration, the mean venous plasma kininogen concentration was 10.6 ± 1.7 µg Bk Eq per ml. This was almost twice ($P<0.01$) the mean control value found in seven healthy volunteers (5.6 ± 1.2 µg Bk Eq per ml).

Table 1: Sequential study of the effect of indomethacin on plasma kininogen and proteins in rheumatoid patients. The table shows the effect of placebo treatment for 48 hrs., followed by treatment for one week with oral indomethacin (50 mg thrice daily) and a final 24 hrs. on placebo. Statistical significance at P 0.05 and P 0.01 respectively of differences from placebo period is indicated by one and two stars. Two patients did not complete the trial.

Parameter	Placebo for 48 hrs.	After start of therapy			Placebo for 24 hrs.
		1 Hr	24 Hrs	1 Week	
Plasma Kininogen (μg Bk Eq/ml)	10.9 ± 1.0	7.0 ± 1.3 **	6.1 ± 0.8 **	5.9 ± 0.4	9.2 ± 1.3
Total Protein g%	7.9 ± 0.5	7.5 ± 0.9	7.5 ± 0.6 *	7.7 ± 0.6	7.6 ± 0.4
α_2-Globulin g%	0.9 ± 0.10	0.78 ± 0.08 **	0.73 ± 0.12 **	0.83 ± 0.17	0.98 ± 0.17
Albumin g%	3.8 ± 0.3	3.7 ± 0.3	3.7 ± 0.2	3.8 ± 0.2	3.7 ± 0.3
N	6	6	6	4	4

Effects of Indomethacin

By 1 hour after first taking indomethacin, the mean kininogen level had fallen to 65% of the placebo level ($P < 0.01$) (Table 1). After 24 hours the level had fallen only another 9% to 56% of the initial level. After 7 days of drug treatment, the indomethacin was once again replaced by placebo and a further 24 hours on placebo caused the mean plasma kininogen to rise again to 9.2 ± 1.3 µg Bk Eq per ml, some 64% above the level found in healthy volunteers.

The speed of the initial fall in plasma kininogen level was noteworthy and in a further study in 6 patients the mean kininogen level had fallen significantly ($P < 0.05$) by 10% only 15 minutes after taking the drug. By 30 minutes, the level had fallen by 34%. Throughout the study the mean haematocrit value was not significantly changed ($P > 0.05$). Table 1 also shows plasma protein levels in these patients. Albumin was unchanged throughout the 8 days regardless of the presence or absence of therapy. The mean total protein fell slightly during the test, but the maximum fall was only 6% at 24 hours following onset of therapy. The mean α_2-globulin level however, was greatly raised at 0.92 ± 0.10g per cent at the start of the test and fell by 15% ($P < 0.01$) one hour after ingesting indomethacin and had fallen maximally by 21% ($P < 0.01$) at 2 hrs.

Of the clinical parameters used to follow the severity of the disease, the joint diameter and grip strength were unaltered by the change from placebo to drug or back again. However, the two forms of pain assessment were dramatically altered by indomethacin therapy. Twenty four hours after starting therapy, both the articular index and the pain index had fallen in every patient, the mean values dropping to half the placebo levels. Twenty four hours after resumption of placebo, the values had returned in every case to their original high levels.

Effects of Aspirin

Table 2 shows plasma kininogen levels in six rheumatoid patients after placebo therapy for 48 hours and then one week after commencing aspirin therapy (975 mg 4 times daily). The plasma kininogen level fell in every case, the mean value falling by 31% ($P < 0.001$). The mean α_2-globulin level fell by 20% while no significant change occurred in the total protein or plasma albumin levels.

Table 3 shows the effect of aspirin on some clinical parameters in these patients. The change from placebo to aspirin

Table 2: Effect of aspirin on plasma proteins in 6 rheumatoid patients. Table shows means ± sd.

	Placebo 48 hrs.	Aspirin 1 week	Fall percent	P (Paired 't')
Kininogen (μg Bk Eq/ml)	9.2 ± 0.7	6.3 ± 0.8	31.5	<0.0005
Alpha-2 Globulin (G per cent)	0.82 ± 0.01	0.65 ± 0.16	20.4	<0.01
Total Protein (G per cent)	7.6 ± 0.5	7.5 ± 0.6	1.5	Not Sign.
Albumin (G per cent)	3.6 ± 0.3	3.4 ± 0.4	4.7	Not Sign.

Table 3: Effect of Aspirin on indices of disease activity in 6 rheumatoid patients. Table shows means ± sd.

		Placebo 48 hrs.	Aspirin 1 week	Percent change	P
Pain Score		3.7 ± 1.8	2.7 ± 1.5	-27.0	< 0.05
Articular Index		21.8 ± 10.9	12.5 ± 4.5	-42.7	< 0.05
Grip Strength (mm μg)	R	112 ± 48	118 ± 55	+ 5.4	NS
	L	105 ± 51	132 ± 49	+25.7	< 0.01
Ring Size (mm)	R	275 ± 14	277 ± 19	+ 0.7	NS
	L	276 ± 17	272 ± 16	- 1.8	NS

therapy caused a marked reduction in both pain score and articular index and the change occurred more often than would be expected by chance ($P < 0.05$). Although the mean left hand grip strength had increased after 7 days of aspirin treatment ($P < 0.01$), neither ring size nor right hand grip strength had changed.

Like indomethacin, the aspirin exerted its action on the plasma kininogen level with remarkable rapidity following an oral dose. Figure (1) shows the plasma kininogen levels in three patients from the aspirin study one and two hours and 1 week after recommencing aspirin therapy. One hour after taking aspirin, the mean plasma kininogen level had fallen by 25%, after two hours the mean level had fallen only another 3%. After one week of aspirin the mean plasma kininogen level had fallen only another 5.6% to 65.6% of the initial raised value.

DISCUSSION

It has been suggested that the components of the kinin system present in synovial fluid are derived mainly from plasma (Jasani, Katori & Lewis, 1969). If this is true, then activation of the synovial kinin system, reported to occur in rheumatoid patients (Melmon et al., 1967; Keele & Eisen, 1970), should produce changes in systemic kininogen levels, possibly stimulating compensatory synthesis of kininogen.

When patients with active rheumatoid arthritis received placebo treatment for 48 hours in the present study, their mean venous plasma kininogen level rose to nearly twice the level in healthy subjects. When placebo was replaced by indomethacin or aspirin therapy, the mean plasma kininogen level fell by nearly half with the former drug and by about a third with the latter drug. The changes were remarkably rapid and commenced within minutes of ingesting the drugs. Our earlier studies showed that these changes are not simple methodological artefacts produced by the effects of the drugs on the kininogen assay (Zeitlin et al, in press). This is borne out by plasma protein measurements in the present study. Little or no change occurred in either total plasma protein or albumin. However, the α_2-globulin fraction of plasma contains kininogen, and in these patients was found to be influenced by the non-steroidal anti-inflammatory drugs in parallel with the changes in plasma kininogen.

Of the clinical indices which we have so far examined, the assessments of pain have so far proved to be the most sensitive to the changes from placebo to drug and back again, marked reduction in pain and a return to high levels of pain respectively occurring within 24 hours. Ferreira and his colleagues have shown that prostaglandins, with little pain-producing activity of their own,

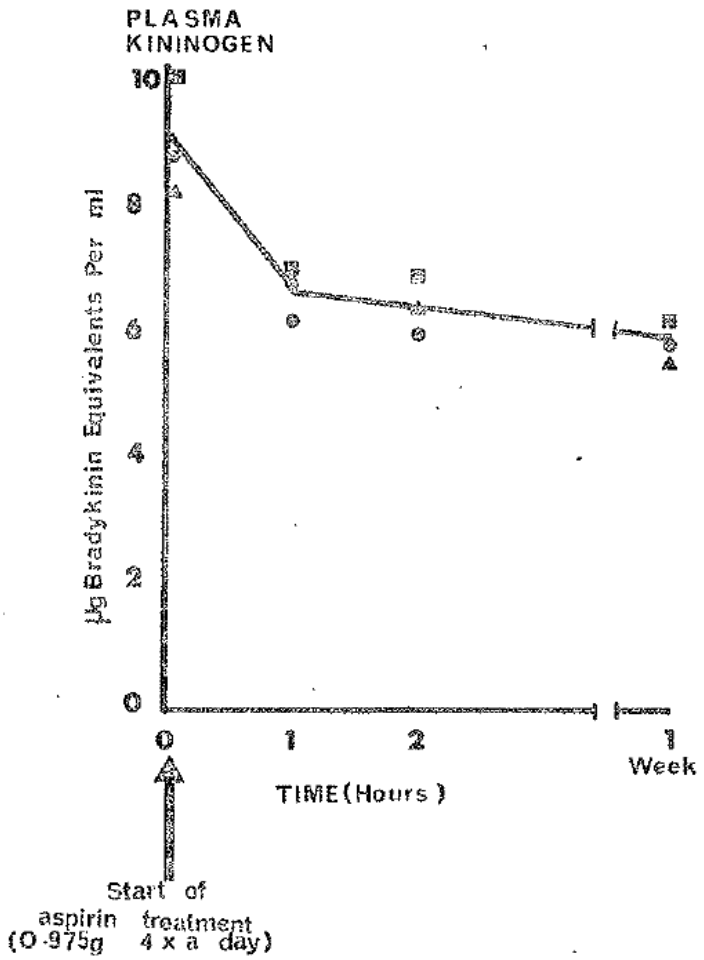

Fig. 1: The effect of aspirin on plasma kininogen in three rheumatoid patients.

powerfully potentiate the pain-producing ability of kinins (Ferreir & Vane, 1975). Indomethacin and aspirin are both powerful inhibito of prostaglandin synthesis (Vane, 1971). The present studies indic that in rheumatoid patients they are also capable of preventing th appearance of raised circulating levels of a kinin precursor in rheumatoid patients.

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